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REVIEW ARTICLE

Neuropilins: expression and roles in the epithelium

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Summary

Initially found expressed in neuronal and then later in endothelial cells, it is well established that the transmembrane glycoproteins neuropilin-1 (NRP1) and neuropilin-2 (NRP2) play essential roles in axonal growth and guidance and in physiological and pathological angiogenesis. Neuropilin expression and function in epithelial cells has received little attention when compared with neuronal and endothelial cells. Overexpression of NRPs is shown to enhance growth, correlate with invasion and is associated with poor prognosis in various tumour types, especially those of epithelial origin. The contribution of NRP and its ligands to tumour growth and metastasis has spurred a strong interest in NRPs as novel chemotherapy drug targets. Given NRP's role as a multifunctional co-receptor with an ability to bind with disparate ligand families, this has sparked new areas of research implicating NRPs in diverse biological functions. Here, we review the growing body of research demonstrating NRP expression and role in the normal and neoplastic epithelium.

Keywords
epithelium, neuropilin, neuropilin-1, neuropilin-2, semaphorin, vascular endothelial growth factor

Neuropilin-1 (NRP1) and neuropilin-2 (NRP2) are transmembrane glycoproteins specific to vertebrates. Originally named A5, NRP1 was first identified by Fujisawa and colleagues in 1987 (Takagi *et al.* 1987) when it was identified as an antigen to a monoclonal antibody which bound to neuronal cell-surface proteins in the optic tectum of *Xenopus* tadpoles. Initially characterized as a neuronal receptor for the class 3 semaphorins (SEMA3), a family of chemorepulsive guidance molecules that repel axons and collapse growth cones, NRP was found to play an essential role in axon growth and guidance. Analysis of mouse chimeras of *NRP1*-overexpressing and *NRP1*-null mutant mice demonstrated that NRP1 was essential for normal embryological development of the nervous and cardiovascular systems (Kitsukawa *et al.* 1995; Kawasaki *et al.* 1999). A decade on from when NRP1 was initially described, NRP2 was identified as an alternative neuronal receptor for certain SEMA3s (Kolodkin *et al.* 1997) with mutant mouse studies revealing that NRP2 has a more restricted role in neuronal patterning (Giger *et al.* 2000) and lymphangiogenesis (Yuan *et al.* 2002). Following the discovery of NRP2, NRPs were identified to be receptors for specific members of the vascular endothelial growth factor (VEGF) family of angiogenic cytokines, following which it soon became apparent that the

NRPs had an important role in physiological and pathological angiogenesis (Staton *et al.* 2007).

Overexpression of NRP1 enhances tumour growth, correlates with invasive growth and is associated with poor prognosis in tumours from the gastrointestinal (GI) tract, prostate, lung, ovary and also gliomas, osteosarcomas and melanomas (Handa *et al.* 2000; Kawakami *et al.* 2002; Klagsbrun *et al.* 2002; Bagri *et al.* 2009). The contribution of NRP and its ligands to tumour growth and metastasis has spurred a strong interest in NRP1 antagonists used in combination with anti-VEGF-chemotherapy as novel anti-angiogenesis therapies (Geretti & Klagsbrun 2007).

Neuropilin's role as a multifunctional co-receptor with an ability to bind with disparate ligand families has sparked new areas of research implicating NRPs in diverse biological functions including T-cell activation (Sarris *et al.* 2008) and viral infection (Jin *et al.* 2010). Neuropilin expression and function in epithelial cells has received little attention when compared with neuronal and endothelial cells. This review will therefore focus on the expression patterns of NRPs and their ligands in epithelial cells, with particular attention to the 'true' epithelium of endodermal origin, which comprises the epithelium of the respiratory, GI and lower urological tracts and also the thyroid, parathyroid and thymus gland. In these organ systems, there is increasing awareness of the physiological and pathological roles of NRPs and their ligands with the potential of NRPs as therapeutic targets.

Neuropilin structure

Neuropilin-1 and NRP2 are 120–130 kDa multifunctional single pass transmembrane glycoproteins with identical domain structures, comprising of a large N-terminal extracellular domain, a short transmembrane domain and a small cytoplasmic domain (Pellet-Many *et al.* 2008). The NRP extracellular region is divided into three domains (Figure 1). Deletion analysis of the domains suggests that the a1/a2 and b1/b2 domains are involved in class 3 semaphorin binding to NRP1 and the b1/b2 is also involved in the binding of $VEGF_{165}$ (Gu *et al.* 2002). Presence of the a1/a2 domain, although not essential, enhances $VEGF_{165}$ binding to NRP1 (Pellet-Many *et al.* 2008). The c- and transmembrane domains are involved in receptor dimerization, a requirement of SEMA 3A signalling, with the c-domain thought to play a role in NRP-1 oligomerization. A neuropilin interacting protein (NIP or synectin) containing cytoplasmic PDZ-domain has also been identified (Cai & Reed 1999). Neuropilins can also exist as soluble isoforms with a naturally occurring soluble NRP1 (sNRP1) first cloned from the human prostate cancer cell line, PC3 (Gagnon *et al.* 2000). Three other sNRP1 species and one sNRP2 species have also been reported (Rossignol *et al.* 2000; Cackowski *et al.* 2004). sNRPs function as natural inhibitors, with sNRP1 acting as a competitive antagonist of $VEGF_{165}$ (Mamluk 2002).

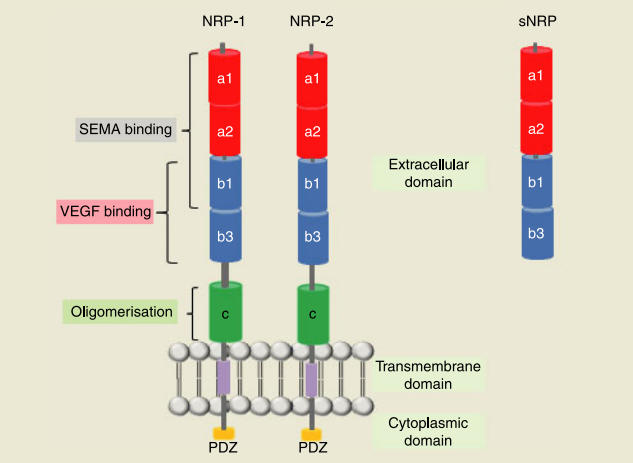

Figure 1 Neuropilin Structure. In humans, Neuropilin-1(*NRP1*) is located on chromosome 10 and *NRP2* on chromosome 2. Despite being located on different chromosomes and NRP2 sharing only 44% sequence homology with NRP1, the two receptors have an identical domain structure, comprising of a large N-terminal extracellular domain (835 aa for NRP1, 844 aa for NRP2), a short transmembrane domain (23 aa for NRP1, 25 aa for NRP2) and a small cytoplasmic domain (44 aa for NRP1, 42 for NRP2). The NRP extracellular region is divided into three domains: (i) the a1/a2 (CUB) domain, which is homologous to complement proteins C1r and C1s, (ii) the b1/b2 domain, which is homologous to coagulation factors V and VIII and (iii) the c domain, which is homologous to meprin, A5 and receptor tyrosine phosphatase μ (hence designated MAM). The PDZ-domain binds the neuropilin interacting protein (NIP). Soluble NRP (sNRP), which contain the extracellular a1/a2 and b1/b2 domains and lack the transmembrane –c and cytoplasmic domains function as natural NRP inhibitors.

Neuropilin ligands and co-receptors

Neuropilins function as co-receptors, binding to extracellular ligands with high affinity and complexing with other transmembrane receptors to form holoreceptors (Pellet-Many *et al.* 2008). Neuropilins have the unusual ability to bind with high affinity to multiple ligand families (Figure 2). It is well established that NRPs are receptors for both the class 3 semaphorins and heparin-binding members of the VEGF family. Recent evidence has revealed that the NRPs may act as receptors for other growth factors in epithelial cells as well.

Semaphorins and plexins

The semaphorins are a large family of transmembrane and secreted proteins. First identified as evolutionary conserved axon-guidance cues (Luo *et al.* 1993), semaphorins are now found to be widely expressed outside the nervous system. Unlike other semaphorins, SEMA3s bind to NRPs as their cell surface receptors. At present, there are seven SEMA3s known, denoted SEMA3A-G (Chen *et al.* 1998). Most of the SEMA3s, with the exception of SEMA3E (Gu *et al.*

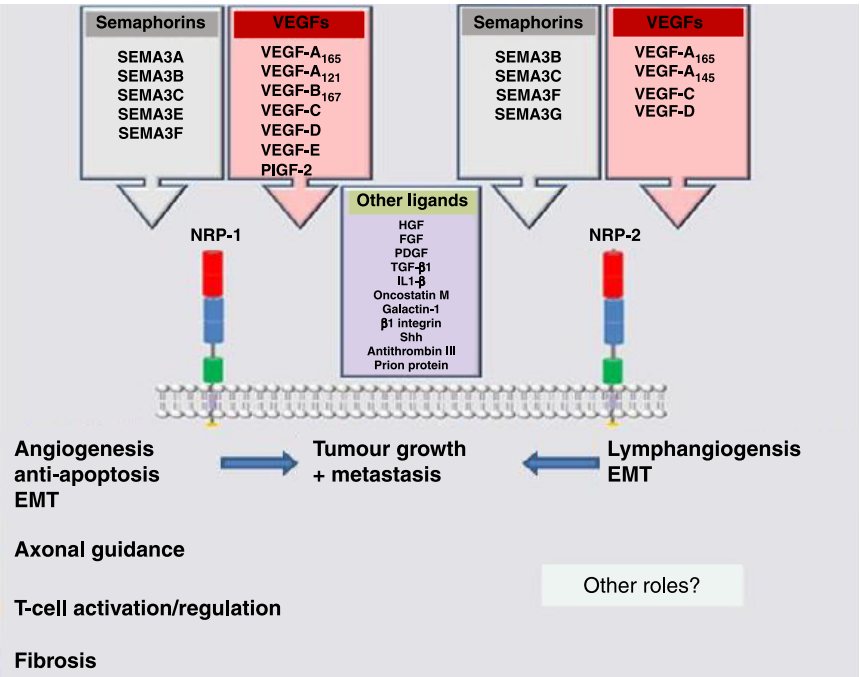

Figure 2 The multiple ligand families of the neuropilins In addition to class III semaphorins and VEGF family, alternative neuropilin (NRP) ligands have been discovered, reflecting NRP promiscuous binding and diverse biological roles.

2005), bind to one of the two neuropilins or to both, with NRP1 primarily responding to SEMA3A (also known as collapsin-1), whereas NRP2 exhibits preferential binding to SEMA3F.

SEMA3s also require interaction with members of the plexin family to signal. Specific plexins, plexin-A1, plexin-A2 (Takahashi & Strittmatter 2001), plexin-A3, plexin-A4 (Yaron *et al.* 2005) and plexin-D1 (Gitler *et al.* 2004; Zhang *et al.* 2009), are known to form complexes with NRPs to transduce the SEMA3 signal, where the NRP serves as the binding receptor and the plexin as the signal-transducing element. It has been proposed that SEMA3A binding results in a 2:2:2 complex between SEMA3A, plexin-A1 and NRP1 (Antipenko *et al.* 2003) (Figure 3a), with the association of plexin-A1 to NRP1 known to increase the affinity of SEMA3A to NRP1 (Neufeld & Kessler 2008). Plexin expression has also been reported in a wide range of epithelial tumours (Syed *et al.* 2005; Nguyen 2006; Wong *et al.* 2007; Zhao *et al.* 2007; Kigel *et al.* 2008).

SEMA3s exert chemorepulsive and anti-angiogenic activity in endothelial cells (Serini *et al.* 2009). In addition to inhibiting VEGF-induced endothelial cell proliferation and migration by inhibiting the binding of VEGF-NRP interaction, SEMA3A and SEMA3F also influence vascular development and angiogenesis by inhibiting integrin-mediated adhesion of endothelial cells to the extracellular matrix and enabling the de-adhesion required for vascular remodelling (Serini *et al.* 2003) and also by inducing endothelial cell apoptosis with the combination of SEMA3A and SEMA3F demonstrating a synergistic effect at high concentrations (Guttmann-Raviv *et al.* 2007). It appears that there is a downregulation of SEMA3 expression with tumour progression (Plotkin *et al.* 2009; Staton *et al.* 2011) with SEMA3s characterized as inhibitors of tumour angiogenesis (Bielenberg *et al.* 2004; Kessler *et al.* 2004). Recent analysis of murine models of multistep carcinogenesis has revealed SEMA3A to be an endogenous anti-angiogenic inhibitor that is present in premalignant lesions and is lost during disease progression where it is associated with an accelerated and chaotic tumour vasculature (Maione *et al.* 2009). This study demonstrates SEMA3A as an anti-angiogenic and anti-tumour drug target where inhibiting endogenous SEMA3A during the angiogenic switch in a pancreatic tumour model enhances angiogenesis and tumour growth. Therapeutic restoration of SEMA3A by somatic gene transfer was also shown to increase pericyte coverage of tumour blood vessels. This key property of tumour vascular normalization provides a potential therapeutic window to optimize the delivery of cytotoxic drugs and also oxygen to sensitize the tumour for radiotherapy (Jain 2005). Data from Maione and colleagues study also suggest that SEMA3A may prolong the duration of this normalization window and therefore provide a wider therapeutic time frame. SEMA3A has also been shown to inhibit the migration of breast cancer cells (Bachelder *et al.* 2003) and invasiveness of prostate cancer cells (Herman & Meadows 2007) *in vitro*. The contribution of the SEMA3A/NRP interaction in some tumour cells may however be more complex, with conflicting reports proposing that SEMA3A may contribute to the progression of carcinoma of the pancreas (Muller *et al.* 2007) and colon (Nguyen 2006). SEMA3B

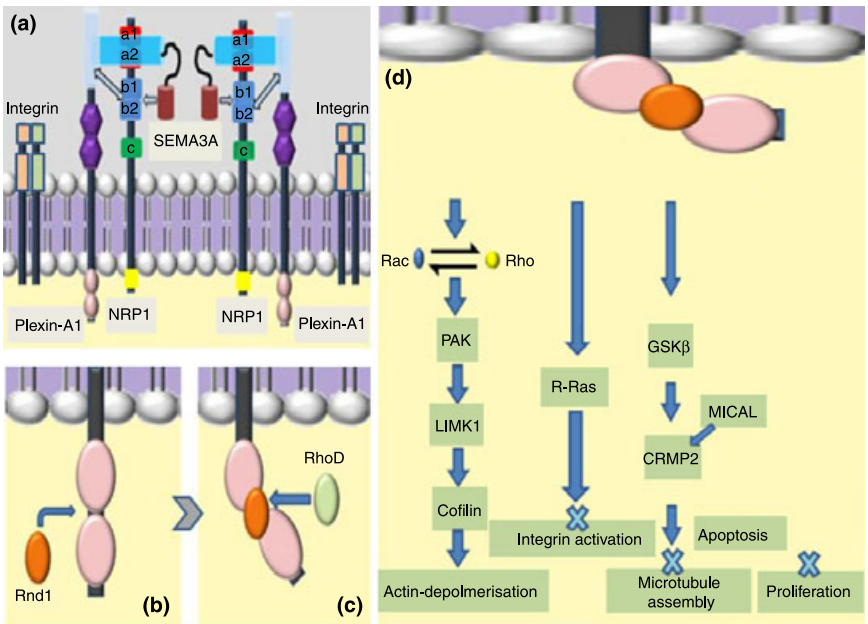

Figure 3 Class 3 semaphorin and neuropilin (NRP) interaction and resulting downstream signalling. (a) SEMA3A consists of a sema domain which interacts with the a1/a2 region of NRP1 and the sema domain of plexin-A, an Ig-like domain and C-terminal base region which interact with the b1/b2 region of NRP1. SEMA3A binding results in a 2:2:2 complex between SEMA3A, plexin-A1 and NRP1. Type-A plexins form complexes with NRPs to transduce the SEMA3 signal, where the NRP serves as the binding receptor and the plexin as the signal-transducing element resulting in neuronal collapse. (b) This is triggered by recruitment of Rnd1 to the cytoplasmic domain of plexin-A1. (c) The plexin-A1 and Rnd1 interaction, which is antagonized by RhoD, results in activation of the plexin intracellular domain. (d) There is a shift in the balance of Rac and Rho activity towards actin depolymerization, through the sequential activation of PAK, LIMK1 and cofilin. Plexin-A1 activation results in R-Ras inactivation which in turn inactivates integrin function, promoting detachment of target cells from the ECM. GSK3-dependent phosphorylation of CRMP2, which binds with MICALS, results in the inhibition of microtubule assembly. Late effects of SEMA3A-NRP signalling lead to the inhibition of ERK phosphorylation, activation of caspases and induction of apoptosis and inhibition of cellular proliferation.

and SEMA3F have been characterized as tumour suppressor genes, inhibiting adhesion, migration and proliferation in cancer cell lines (Tomizawa *et al.* 2001; Bielenberg *et al.* 2004; Nasarre *et al.* 2005) and are regulated by the p53 tumour suppressor gene. SEMA3D and SEMA3G may also possess anti-tumourogenic and anti-angiogenic properties (Kigel *et al.* 2008).

VEGF and VEGF receptors

Originally discovered as a potent 'vascular permeability factor' (VPF) (Senger *et al.* 1986), VEGF is a potent angiogenic, vasoactive molecule which increases vascular permeability and acts as a endothelial cell chemotactic, survival and proliferation factor (Bates & Harper 2002; Jain 2003). With their tyrosine kinase receptors, VEGF-receptor-1 (VEGFR-1), VEGF-receptor-2 (VEGFR-2) and VEGF-receptor-3 (VEGFR-3), the VEGF family have a vital role in physiological and pathological angiogenesis (Staton *et al.* 2007). Of the multiple VEGF isoforms, $VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$ predominate, with $VEGF_{165}$ the most abundant, active and studied (Staton *et al.* 2007). Overexpression of VEGF has been detected in almost all human cancers investigated. Higher serum levels of VEGF correlate with advanced disease in colon cancer (Takahashi *et al.* 1995; Galizia *et al.* 2004) and poor prognosis in gastric cancer (Maeda *et al.* 1998). More recently, it appears VEGF may act as an internal autocrine survival factor in NRP positive tumour cells (Lee *et al.* 2007; Barr *et al.* 2008). As well as regulating angiogenesis, VEGF is considered a potent growth factor for epidermal tumours (Lichtenberger *et al.* 2010).

Soker *et al.* (1998) first described NRP1 as a functional receptor for specific members of the VEGF family of angiogenesis factors with NRP2 subsequently discovered to be a receptor for VEGF (Gluzman-Poltorak *et al.* 2000). Unlike VEGFRs, NRP does not have a tyrosine kinase domain and therefore acts a co-receptor for $VEGF_{165}$. Neuropilin-1 is therefore a co-receptor for VEGFR-2, with $VEGF_{165}$ able to bind to both NRP1 and VEGFR-2 simultaneously (Figure 4). Soker *et al.* (1998) demonstrated that co-expression of NRP1 with VEGFR-2 enhanced $VEGF_{165}$-mediated chemotaxis with NRP1 enhancing both VEGFR-2 binding and bioactivity. Neuropilin-2 is a co-receptor for VEGFR-3 with co-localization of NRP2 with VEGFR-3 demonstrated when stimulated by VEGF-C and VEGF-D (Karpanen *et al.* 2006). These two VEGF polypeptides have also been shown to induce lymph vascular development and stimulate lymph node metastasis via VEGFR-3 in mouse models (Lohela

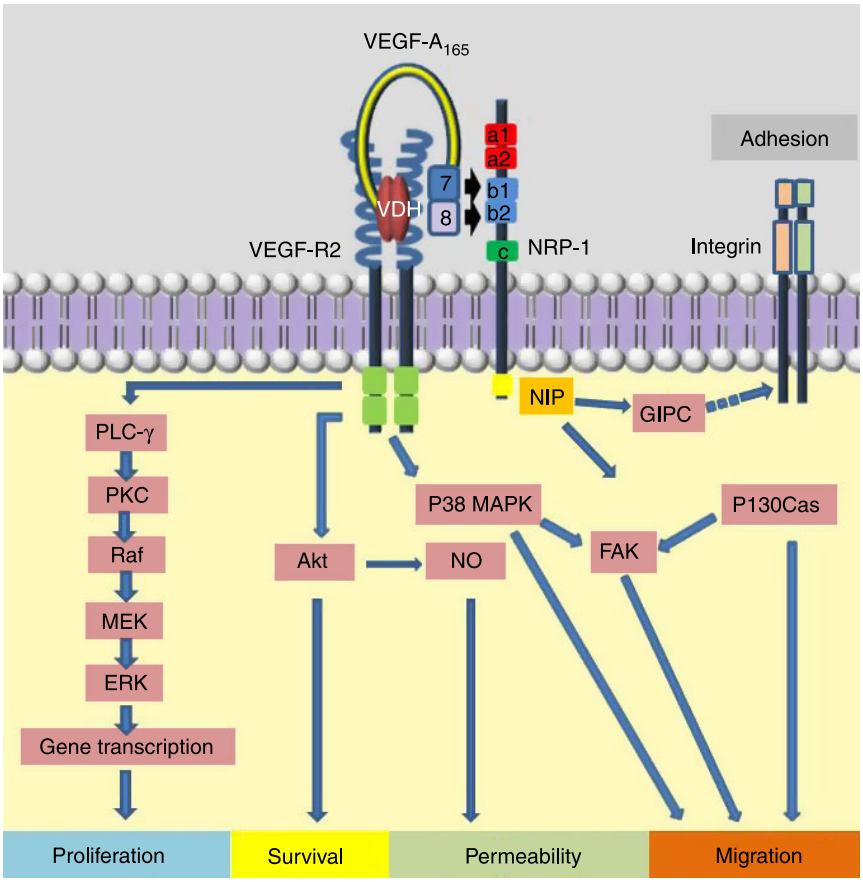

Figure 4 Vascular endothelial growth factor (VEGF) interaction with VEGF-R2 and neuropilin-1 (NRP-1) and downstream signalling. VEGF-A_{165} interacts with VEGF-R2 via the vascular homology domain (VDH) and with the b1 domain of NRP-1 via exons 7 and 8. Binding to the b2 region of NRP-1 contributes to optimal binding. Cellular proliferation, migration, survival and vascular permeability result via downstream signalling initiated by VEGR-2 tyrosine phosphorylation and activation of multiple phosphorylated signalling molecules. Neuropilin interacting protein (NIP) participates in protein scaffolding to regulate actin cytoskeletal dynamics, cell migration, invasion and adhesion.

et al. 2009), further explaining NRP2's role in lymphangiogenesis.

Other ligands and co-receptors

Given their promiscuous binding and the suggestion that NRPs may interact with other heparin-binding proteins from outside the VEGF family, more novel NRP ligands have been discovered (Figure 2). It is now known that NRPs bind to members of the fibroblast growth factor (FGF) family (FGF-1, FGF-2, FGF-4) (West *et al.* 2005) as well as galectin-1(Hsieh *et al.* 2008), hepatocyte growth factor/scatter factor (HGF/SF) (West *et al.* 2005; Hu *et al.* 2007; Matsushita *et al.* 2007), anti-thrombin III, prion protein (West *et al.* 2005), transforming growth factor-β1 (TGF-β1), and platelet-derived growth factor (PDGF) (Ball *et al.* 2010).

Fibroblast growth factor-2 binds with NRP1, stimulating the growth activity of the ligand on human umbilical vein endothelial cells (HUVECs) (West *et al.* 2005; Matsushita *et al.* 2007) and galectin-1, a carbohydrate-binding protein, selectively binds to NRP1, via the carbohydrate-recognition domain. The Gal-NRP1 interaction mediates endothelial cell migration and adhesion and enhances VEGFR-2 phosphorylation in oral squamous cell carcinoma (Hsieh *et al.* 2008). Banerjee *et al.* (2006) demonstrated that PDGF, derived from breast cancer cells, also interacts with NRP1, promoting motility in vascular smooth muscle cells.

Neuropilins may also interact with other cellular receptors, for example, NRP1 has been shown to complex with β1 integrin in pancreatic cancer cell lines, and the ectoprotein kinase, CK2, appears to interact with and phosphorylate the NRP1 extracellular domain (Shintani *et al.* 2009). c-Met, a tyrosine kinase receptor that binds HGF (Jiang *et al.* 2005), also interacts with NRP1. Hepatocyte growth factor/c-met signalling plays a vital role in the development and regeneration of several organ systems (Birchmeier *et al.* 2003) and regulation of endothelial cell survival, proliferation and migration (Ding *et al.* 2003). Recent studies have demonstrated that NRP1 and NRP2 act as a functional co-receptor for HGF, enhancing HGF/c-met binding and

leading to increased tumour invasiveness (Hu *et al.* 2007; Matsushita *et al.* 2007; Sulpice *et al.* 2008).

Recent work has demonstrated that NRP also influences TGF-β1 signalling. Neuropilin-1 has been found to be a receptor for the latent and active forms of TGF-β1, where it activates latent TGF-β1 and promotes regulatory T-cell activity (Glinka & Prud'homme 2008). TGF-β1 is established as a master regulator of epithelial mesenchymal transition (EMT) (Zavadil & Bottinger 2005) with *in vitro* studies demonstrating TGF-β1 induction of EMT in certain types of cancer cells (Wendt *et al.* 2009). Epithelial mesenchymal transition is the process whereby molecular alterations to epithelial cells promote dysfunctional cell–cell adhesive interactions and junctions, thereby promoting cancer cell progression and invasion into the surrounding microenvironment (Kalluri & Weinberg 2009). Neuropilin's role in EMT and also in organ fibrosis has attracted more interest of late. It has been found that VEGF and NRP-1 directly promote EMT (Mak *et al.* 2010). Treatment of prostate cancer cell lines (PC3) with recombinant $VEGF_{165}$ resulted in decreased E-cadherin with a fusiform morphology and increased expression of N-cadherin and vimentin. Utilizing shRNA, NRP1 knockdown PC3 cells were found to be resistant to TGF-β1 treatment compared with control cells as evidenced by their morphology and expression of EMT markers. *In vitro* work by Mak and colleagues had led to the proposal that the VEGF/NRP1 pathway may be regulated by the oestrogen receptor beta (ERβ1). Interaction of ERβ1 with its ligand 3*β*-Adiol represses hypoxia-inducible factor-1 (HIF-1)-mediated VEGF-A transcription and therefore represses EMT via NRP1. siRNA targeting of NRP2 on colorectal cancer cells treated with pharmacological inhibitors of TGF-β1 type I receptor *in vitro* has also been shown to promote EMT (Grandclement *et al.* 2011).

A role for NRP in fibrosis has also been proposed, with NRP1 found to enhance TGFβ1 and PDGF signalling – in hepatic stellate cells and thereby promoting liver fibrosis (Cao *et al.* 2010a). Schramek *et al.* (2009) have also investigated the effect of pro-fibrotic cytokines on NRP expression in human proximal tubular cells. TGF-β1 and interleukin-1β (IL-1β) induced upregulation of NRP2 expression but, contrary to other reports, a downregulation of NRP-1 expression. Oncostatin M (OSM), on the other hand, stimulated the expression of both NRP-1 and NRP2. Both of these studies are described in more detail in the respective sections later in this article.

Recent work also demonstrates that NRPs are positive regulators of Hedgehog (Hh) signal transduction (Hillman *et al.* 2011). Hedgehog signalling is critical during embryogenesis and in adult tissue, including the development of the GI tract, and contributes to cellular differentiation, proliferation and maintenance (McMahon *et al.* 2003). Dysregulation of sonic hedgehog signalling (Shh), the best studied ligand of the Hh signalling pathway, has been implicated in the development of various cancers, including those of the oesophagus, stomach, pancreas, colon and kidney (Saqui-Salces & Merchant 2010). There is evidence that Shh dysregulation is an early event in colon cancer carcinogenesis (Yoshikawa *et al.* 2009). It has been shown previously that NRP1 may be a target for Shh signalling (Hochman *et al.* 2006) with VEGF under the transcriptional control of the Shh pathway (Dormoy *et al.* 2009). Cao *et al.'s* (2008) study also strongly suggests that NRP-1 knock-down promotes renal cancer cell differentiation due in part to an inability to express Shh. Targeting Shh in cancer therapy, including metastatic colon cancer, is now the focus of Phase II clinical trials (De Smaele *et al.* 2010). In the normal colon, Shh is expressed at the base of the crypts (Oniscu *et al.* 2004), which is also where NRP1 expression has been noted. Hepatocyte growth factor, FGF, FGFR and TGF-α are also expressed in normal colonic epithelium, with intestinal endocrine cells expressing FGF and TGF-α. These studies suggest the NRPs have functions independent of their conventional ligands, and it is anticipated the NRPs may have a far wider spectrum of activity than is currently appreciated.

Signalling pathways consequent on neuropilin ligation

A recent review has highlighted current knowledge of the signalling pathways arising from NRP (Zachary *et al.* 2009) with growing evidence indicating that selective NRP-mediated signalling takes place via its cytoplasmic domain modulating intracellular signalling through protein–protein interactions (Wang *et al.* 2006). In neuronal cells, the cytoplasmic domain of plexins is responsible for the downstream signalling induced by semaphorins that results in cytoskeletal collapse of neurones (Figure 3). In the absence of a ligand, plexins assume an auto-inhibited state. The formation of the NRP1-SEMA3A-plexin-A1 complex results in a conformational change in the plexin-A1 with relief of auto-inhibition. This results in activation of the plexin intracellular domain and initiation of chemorepellent signal transduction that results in neuronal collapse. This is triggered by recruitment of the small GTPase Rnd1 to the cytoplasmic plexin-A1 (Figure 3b). The plexin-A1 and Rnd1 interaction, which is antagonized by RhoD (Figure 3c), results in activation of the plexin intracellular domain and downstream signalling events that shift the balance of Rac and Rho activity towards actin depolymerization, through the sequential activation of p21-activated kinase (PAK), LIM kinase 1 (LIMK1) and the actin-binding factor cofilin. Activation of plexin-A1 also results in R-Ras inactivation (Oinuma *et al.* 2004). R-Ras regulates integrin function, and its inactivation in turn leads to inactivation of integrin-*β*1 which promotes detachment of the target cell from the extracellular matrix. Integrin inactivation may represent an important anti-tumorigenic mechanism of SEMA3s. The SEMA3-Plexin-A interaction has also been shown to lead to GSK3-dependent phosphorylation of collapsin response mediator proteins (CRMPs), such as CRMP2 resulting in the inhibition of microtubule dynamics and the organization of the actin cytoskeleton (Neufeld & Kessler 2008).

Plexin-A1 activation also leads to the activation of MICALS (molecules interacting with CasL), which form com-

plexes with CRMPs and disassemble both individual and bundled F-actin (Hung & Terman 2011). It has also been observed that prolonged stimulation by SEMA3s can induce apoptosis of endothelial and neuronal cells (Shirvan *et al.* 1999; Guttmann-Raviv *et al.* 2007) (Figure 3d).

Multiple phosphorylated signalling molecules have been implicated in mediating the diverse biological functions following VEGF ligation (Zachary 2003) (Figure 4). Downstream signalling initiated by VEGR-2 tyrosine phosphorylation involves activation of protein kinase C (PKC) and the RAF-mitogen-activated protein kinase (MAPK)/ERK pathway, Akt1, focal adhesion kinase (FAK) and phospholipase-C-γ (PLC-γ). Nitric oxide (NO) and prostaglandins are also involved in linking these postreceptor signalling cascades to biological function. The NRP cytoplasmic domain was initially thought too small to transduce biological signals; however, Wang *et al.* (2006) identified that this intracellular domain is required for NRP-mediated angiogenesis via G-protein signalling molecules. Neuropilin interacting protein, in the PDZ-domain, is thought to be involved in regulating arterial branching morphogenesis and interacting with GTPase-activating protein providing a NRP1-mediated signal transduction. Findings suggest that NIP participates in protein scaffolding, with NIP interacting with up to 20 other proteins (Abramow-Newerly *et al.* 2006) including integrin subunits, RGS19 or GAIP, and Rho-GEF or syx1 (Liu & Horowitz 2006). Valdembri *et al.* (2009) have demonstrated that NRP1 promotes endothelial cell adhesion to the extracellular matrix protein fibronectin by regulating α5β1 integrin traffic. Neuropilin-1's short cytoplasmic domain binds with the adaptor protein GIPC1 which results in integrin internalization leading to cell adhesion to fibronectin, essential for embryonic vascular development and tumour angiogenesis (Hynes 2007). Neuropilin interacting protein therefore acts as a link between the surface receptors, integrins and downstream intracellular signalling molecules which then regulate actin cytoskeletal dynamics, cell migration, invasion and adhesion. Neuropilin-1 has also been found to mediate the phosphorylation of the adaptor and actin cytoskeletal associated protein p130Cas (Evans *et al.* 2011) which is involved in cytoskeletal reorganization where it interacts with FAK. Increased p130Cas tyrosine phosphorylation has been found to result in an increase in cell invasion (Defilippi *et al.* 2006). The VEGF/NRP1 interaction also influences p38MAPK activation and the formation of pericyte-associated vessels (Kawamura *et al.* 2008). An alternative signalling pathway hypothesized, whereby NRP1 recruits specific signalling networks to the VEGF/VEGFR-2 axis rather than a NRP1/VEGFR-2 complex being required to optimize signalling through VEGFR-2 (Zachary Ian *et al.* 2009) remains untested.

Although VEGFR-2 tyrosine phosphorylation can be induced by VEGF independent of NRP1 (Waltenberger *et al.*

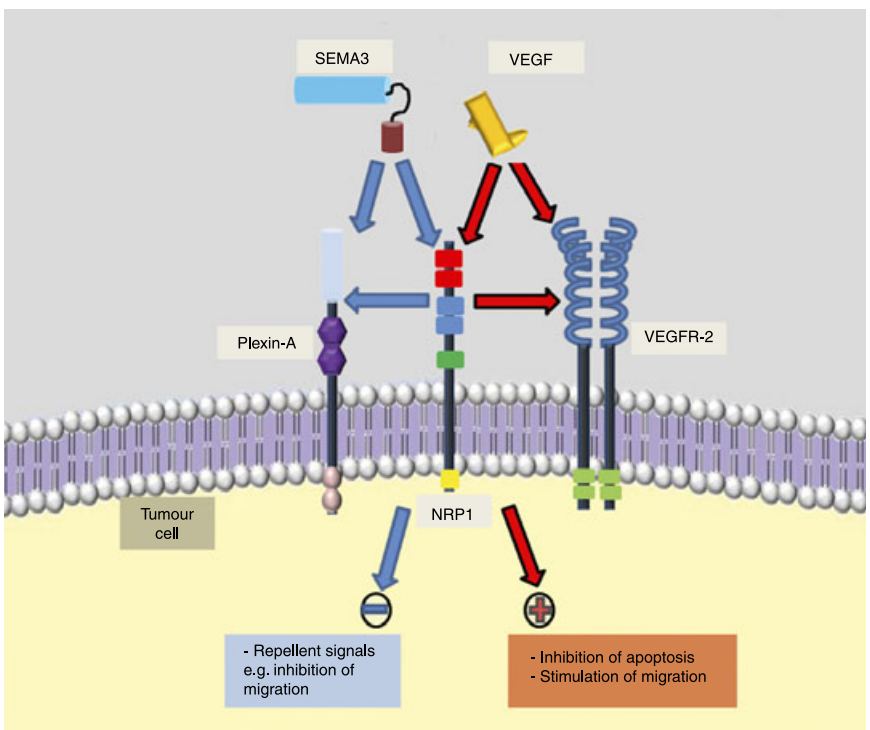

Figure 5 Class 3 Semaphorins (SEMA3) compete with vascular endothelial growth factor (VEGF) for binding to Neuropilins (NRPs) in tumour cells. This leads to dimerization and interaction with plexin-A, in turn leading to intracellular signalling, causing inhibition of tumour cell migration. VEGF binds to NRP1 which dimerizes and causes intra-cellular signalling, directly through neuropilin interacting protein (NIP) or VEGF-R2 if present, causing inhibition of apoptosis and stimulation of tumour cell migration.

Table 1 Summary of the epithelial tumours expressing NRP1 and NRP2

Tumour	NRP1	NRP2	Reference
Oesophageal carcinoma	✔	–	Hansel *et al.* (2004)
Gastric carcinoma	✔	–	Akagi *et al.* (2003)
	✔	–	Hansel *et al.* (2004)
Colorectal carcinoma	✔	–	Parikh *et al.* (2004)
	✔	–	Hansel *et al.* (2004)
	✔	–	Ochiumi *et al.* (2006)
	✔	–	Kamiya *et al.* (2006)
	–	✔	Gray *et al.* (2008)
GI carcinoid tumours	–	✔	Cohen (2001)
Pancreatic neuroendocrine tumour	–	✔	Cohen *et al.* (2002)
Pancreatic carcinoma	✔	–	Parikh *et al.* (2003)
	✔	–	Hansel *et al.* (2004)
	✔	✔	Fukahi *et al.* (2004)
	✔	✔	Li *et al.* (2004)
	✔	–	Muller *et al.* (2007)
Hepatocellular carcinoma	✔	–	Raskopf *et al.* (2010)
	✔	–	Berge *et al.* (2011)
Cholangiocarcinoma	✔	–	Hansel *et al.* (2004)
Transitional cell carcinoma of bladder	✔	–	Sanchez-Carbayo *et al.* (2003)
Prostate carcinoma	✔	–	Latil *et al.* (2000)
	✔	–	Vanveldhuizen *et al.* (2003)
	✔	–	Yacoub *et al.* (2009)
Lung carcinoma	✔	✔	Kawakami *et al.* (2002)
	✔	✔	Lantuejoul *et al.* (2003)
Laryngeal carcinoma	–	✔	Zhang & Kong (2006)
Breast carcinoma	✔	–	Stephenson *et al.* (2002)
	✔	–	Bachelder *et al.* (2003)
	✔	–	Ghosh *et al.* (2008)
	–	✔	Yasuoka *et al.* (2009)
	✔	✔	Staton *et al.* (2011)
Ovarian carcinoma	✔	–	Hall *et al.* (2005)
	✔	✔	Osada *et al.* (2006)
	✔	–	Baba *et al.* (2007)
	✔	–	Drenberg *et al.* (2009)
Cutaneous melanoma	✔	✔	Lacal *et al.* (2000)
	✔	–	Straume & Akslen (2003)
	–	✔	Rushing *et al.* (2011)
Papillary carcinoma of thyroid	–	✔	Finley *et al.* (2004)
Salivary adenoid cystic carcinoma	–	✔	Cai *et al.* (2010)
Retinal pigment epithelium	✔	–	Cui *et al.* (2003)
	✔	–	Lim *et al.* (2005)

1994), further evidence (Whitaker *et al.* 2001; Pan *et al.* 2007; Kawamura *et al.* 2008) indicates that NRP1 is required for optimal VEGF/VEGFR-2 signalling and for specific function, such as migration, rather than for all VEGF-induced biological responses.

In tumour cells, SEMA3s are thought to compete with VEGF for binding with NRPs. Figure 5 illustrates the SEMA3 and VEGF interaction with NRPs and the effects on tumour cell biology. Miao *et al.* (2000) proposed the theory that VEGF may bind to NRP-1 on tumour cells and VEGFR-2 on endothelial cells simultaneously increasing endothelial cell activity and providing a juxtacrine mechanism for NRP1 induction of angiogenesis and tumour growth.

Neuropilins, their ligands and co-receptors in epithelial cells

When compared to the normal epithelium, NRP expression in the neoplastic epithelium is more widely described. Although NRP1 and NRP2 are often co-expressed, (Pellet-Many *et al.* 2008) NRP1 is predominantly expressed in carcinomas (tumours of epithelial cell origin). In comparison, neoplasms that are not of epithelial origin, such as melanomas, neuroblastomas and glioblatomas, express less NRP1 (Bielenberg *et al.* 2006) (Table 1). Recent research focussed on non-neoplastic epithelium has also implicated NRP in various physiological and pathological processes (Table 2).

Table 2 Summary of the novel biological roles of NRP in different non-neoplastic epithelial cells

Organ system	Epithelium type	Function/role
Upper GI tract	Intestinal epithelium	NRP2 expressed in gastric and small intestine serotonin producing enteroendocrine cells (Cohen 2001)
Pancreas	Pancreatic islet epithelium	Pancreatic islet neogenesis Development of type I diabetes in children (Hasan *et al.* 2010)
Hepato-biliary	Hepatic stellate cells	Increased expression of NRP following partial hepatectomy (Braet *et al.* 2004) NRP expression correlates with severity of hepatic fibrosis (Cao *et al.* 2010a)
Lower GI tract	Intestinal epithelium	Colocalises with enteroendocrine cell subpopulation (Cohen 2001; Yu *et al.* 2011) Possible role in the colonic response to butyrate (Yu *et al.* 2010)
Urinary tract	Renal glomerular epithelium (podocytes)	Glomerulogenesis (Robert *et al.* 2000), maintenance of glomerular filtration barrier (Harper *et al.* 2001) Potential marker for immune status of renal graft (Zhou *et al.* 2007) Decreased expression in diabetic nephropathy (Zhou *et al.* 2007)
	Bladder urothelium	Chronic bladder inflammation/interstitial cystitis (Saban *et al.* 2008a; Cheppudira *et al.* 2008)
Respiratory tract	Alveolar epithelium	Lung organogenesis (Roche *et al.* 2002), lung branching (Ito *et al.* 2000; Kagoshima & Ito 2001) Homeostasis of normal alveolar epithelium (Le *et al.* 2009) Reduced expression in COPD (Marwick *et al.* 2006)
Epidermis	Keratinocytes	Autocrine signalling role in epidermis (Man *et al.* 2006) Wound repair (Kumar *et al.* 2009) Increased expression in psoriasis (Detmar *et al.* 1994; Henno *et al.* 2010)
Thymus	Thymic epithelial cell (Dendritic cells)	T-cell activation [13] (Takamatsu *et al.* 2010) Sema3A-mediated thymocyte migration (Lepelletier *et al.* 2007)
Retina	Retinal pigment epithelium	Choroidal neovascularisation in age-related macular degeneration (Cui *et al.* 2003; Lim *et al.* 2005)

Gastrointestinal tract

Initial work demonstrating NRP1 expression (Parikh *et al.* 2003, 2004; Hansel *et al.* 2004) and NRP2 expression (Cohen 2001) in the normal and neoplastic epithelium has provided a platform on which a number of studies investigating NRP's role in the GI tract have emerged. Furthermore, expression of NRP's co-receptors and ligands has been demonstrated, especially VEGF, where robust expression is seen in almost all digestive tract carcinomas (Brown *et al.* 1993).

Upper GI tract. Although, to date, NRP1 expression has not been demonstrated in the normal oesophageal epithelium, and likewise in early precursor lesions of oesophageal cancer (Barrett's oesophagus and low-grade dysplasia), NRP1 expression has been observed in high-grade oesophageal dysplasia in mucosa adjacent to invasive cancer (Hansel *et al.* 2004). Invasive adenocarcinoma of the oesophagus demonstrated a high NRP1 expression, as did liver and lung metastases from primary oesophageal lesions (Hansel *et al.* 2004). Neuropilin-2-expressing cells have been demonstrated in metaplastic mucosa in Barrett's oesophagus (Cohen 2001); however, there are no reports of NRP2 expression in normal or invasive cancer cells of the oesophagus. Cohen *et al.'s* immunohistochemical analysis has, however, demonstrated NRP2 expressing enteroendocrine cells in the normal stomach and small intestine with NRP2 staining concentrated in vesicle-like structures located near the nucleus at the basolateral side of the serotonin-producing enteroendocrine cells (Cohen 2001).

In keeping with its role in angiogenesis, NRP has been found to be expressed in gastric cancer micro-vessel endothelial cell lining (Kim *et al.* 2009), with overexpression of NRP2 significantly increasing proliferation and migration induced by VEGF. Neuropilin-1 expression has also been demonstrated in gastric tumour epithelial cells in 8 out of 10 specimens, with co-localization of NRP1 and epidermal growth factor receptor (EGF-R) in one-third of differentiated and one-half of undifferentiated cancers (Akagi *et al.* 2003). In the same study, 5 out of 7 gastric cancer cell lines expressed NRP1 mRNA, with an upregulation of NRP1 and also VEGF mRNA expression in response to EGF treatment, suggesting a role for EGF and EGF-R in the regulation of NRP-1 and VEGF expression in gastric cancer.

Pancreas. Although first thought that pancreatic ductal cells did not express NRP1 unless they become tumorigenic (Parikh *et al.* 2003), sparse expression of NRP1 has since been observed in normal ductal epithelium (Hansel *et al.* 2004). Recently, Hasan *et al.* (2010) also demonstrated NRP1 expression confined to the islets cells of normal human pancreas tissue, with co-localization to anti-insulin and anti-glucagon staining cells. Neuropilin-2 is also expressed in the normal pancreas (Li *et al.* 2004), with immunostaining demonstrating expression in a distinct subset of islet cells situated at the periphery of the islet (Cohen *et al.* 2002). An association with minor alleles of two single nuclear polymorphisms on the NRP1 gene and type I diabetes in children has also been discovered (Hasan *et al.* 2010). With VEGF signalling previously implicated in pancreatic islet

neogenesis, this has led to speculation that NRPs could influence the development of some cases of type 1 diabetes in children, by enhancing VEGF-mediated islet cell regeneration, and thus delay onset of the disease.

Parikh *et al.* (2003) first reported NRP1 expression in pancreatic adenocarcinomas, with immunofluorescence staining demonstrating localization of NRP1 to the adenocarcinoma epithelium. In this study, NRP1 expression was upregulated by EGF but not by tumour necrosis factor-α (TNFα). Neuropilin-1 labelling has also been identified in metaplastic pancreatic ductal epithelium, with a dramatic upregulation of NRP1 protein expression in pancreatic adenocarcinoma (Hansel *et al.* 2004). Overexpression of NRP2 is also seen in pancreatic adenocarcinomas (Fukahi *et al.* 2004) and neuroendocrine tumours of the pancreas (Cohen *et al.* 2002). By transfecting the pancreatic cancer cell line PANC-1 with NRP1 antisense cDNA, Fukasawa *et al.* (2007) demonstrated decreased growth, adhesion and invasiveness of cancer cells, indicating that NRP1 confers a growth and survival advantage in pancreatic cancer. Contradicting results were, however, obtained by Gray *et al.* (2005) where overexpression of NRP1 in PANC-1 was shown to decrease cell growth and migration *in vitro* and reduce tumour size *in vivo*, which suggests a more complex role of NRP1 in the growth regulation of tumour cells.

SEMA3A and VEGF are both overexpressed in pancreatic carcinoma, with SEMA3A expression associated with poor prognosis (Muller *et al.* 2007) and the results of a meta-analysis supporting the immunohistochemical expression of VEGF as a prognostic marker in resected pancreatic cancer (Smith *et al.* 2011). However, new evidence has emerged of alternative signalling pathways involving NRP1 and pancreatic cancer. Li *et al.* (2004) revealed an absence of VEGFR expression in resected pancreatic carcinoma specimens that expressed NRP1. Likewise, VEGFR mRNA was not detected in PANC-1 cells, and with exogenous VEGF significantly increasing cellular proliferation, this suggests that NRP1 may mediate pancreatic cancer cell growth in an autocrine mechanism, independent of VEGFR.

It has also been demonstrated that NRP1 complexes with integrin β1 in PANC-1 (Fukasawa *et al.* 2007). Integrins are cell adhesion receptors that regulate a diverse range of cellular functions crucial to the initiation, progression and metastasis of solid tumours (Desgrosellier & Cheresh 2010), and NRP1 interaction with integrin β1 may mediate signalling events that promote cell adherence and invasiveness. The pro-oncogenic molecule interleukin-6 (IL-6) increases the expression of $VEGF_{165}$ and NRP1 in pancreatic cancer cells (Feurino *et al.* 2007), whilst interleukin-8 (IL-8), which is overexpressed in most human pancreatic cancer cell lines, also upregulates $VEGF_{165}$ and both NRP1 and NRP2 in BxPC-3 pancreatic cancer cells (Li *et al.* 2008). In addition, it has been observed that NRP1 complexes with c-Met, a tyrosine kinase receptor that binds HGF, with NRP1 overexpression associated with enhanced cell invasiveness in pancreatic cell lines in response to HGF (Matsushita *et al.* 2007).

With increased interest of NRP1 as a novel target in pancreatic cancer (Matsushita *et al.* 2010; Muders 2011), NRP1 may also contribute to chemoresistance in pancreatic adenocarcinoma, with overexpression of NRP1 in pancreatic cancer cell lines shown to enhance cell survival following growth in suspension and exposure to the chemotherapeutic agents gemcitabine and 5-FU (Wey *et al.* 2005). shRNA-NRP2 transfection reduces NRP2 expression in PDAC cells, leading to decreased survival, migration and invasion *in vitro* and reduced tumour growth *in vivo*, also suggesting NRP2 as a potential therapeutic target.

Hepato-biliary tract. Neuropilin-1 and NRP2 expression has not been detected in normal hepatocytes, but NRP1 has been identified in hepatic stellate cells and in liver sinusoidal endothelial cells (Cao *et al.* 2010b), where expression increases following partial hepatectomy under shear stress conditions (Braet *et al.* 2004). In a rodent model, NRP1 expression correlates with the severity of fibrosis in non-alcoholic steatohepatitis and hepatitis C, with NRP1 enhancing hepatic stellate cell (HSC) migration and TGFβ1-dependent collagen production in human HSC cell lines (Cao *et al.* 2010a). In this study, NRP1 was found to co-localize with PDGF-receptorβ1 in HSCs. It was also observed that NRP1 enhances PDGF binding to PDGF-receptorβ1, with NRP1 complexing with the non-receptor kinase c-Abl to achieve this effect. This data suggest a role for NRP1 in normal liver function.

Neuropilin-1 is expressed in hepatocellular carcinoma (HCC) (Raskopf *et al.* 2010) with increased expression demonstrated in human tumour hepatocytes (Berge *et al.* 2011). Hansel *et al.* (2004) also demonstrated upregulation of NRP1 in ampullary and cholangiocarcinomas, with NRP1 expression significantly increased in invasive versus dysplastic lesions. Berge *et al.* (2011) provided further insight into the role of NRP1 in HCC growth by demonstrating that increased expression of NRP1 in both the vascular and tumour compartments in the liver of transgenic HCC mice corresponds with disease progression. Furthermore, blocking NRP1 function with peptide N (an anti-angiogenic recombinant protein that binds NRP1 and inhibits VEGF-A165/NRP1 interaction) (Sulpice *et al.* 2008) leads to inhibition of tumour liver growth, highlighting the possibility of therapeutically targeting NRP1 for the treatment of HCC.

Lower GI tract. Both NRP1 and NRP2 are expressed in normal colonic epithelium at both m-RNA and protein level (Cohen 2001; Hansel *et al.* 2004). In non-neoplastic colonic epithelium, focal expression of NRP1 and NRP2 has been demonstrated predominantly at the lateral and apical surfaces of the colonic crypts with the distribution and morphology of NRP positive cells in normal colon and appendix thought to mirror that of enteroendocrine cells (Figure 6). Immunohistochemical analyses have, however, demonstrated partial co-localization of NRP1 (Yu *et al.* 2011) and NRP2 (Cohen 2001) with cells that express chromogranin-A (CgA), a general marker of enteroendocrine cells. Interestingly, it has

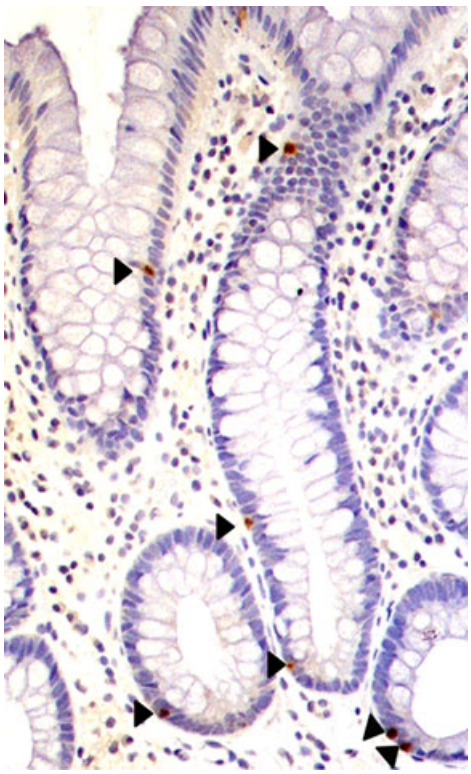

Figure 6 Neuropilin-1 (NRP-1) expression in non-neoplastic epithelial cell of the colon. Immunohistochemical staining of normal human colonic epithelium demonstrating singly dispersed NRP-1 positive cells on the lateral and apical surfaces of the colonic crypts. As well as an expression pattern that mirrors enteroendocrine cells (EEC), the morphology of NRP1 staining cells is similar, with relatively small nuclei and basally orientated cytoplasm often without obvious continuity with the lumen (×40 objective lens, Author's own photograph [JW]).

been demonstrated (Gulubova and Vlaykova 2008), that Cg-A. that Cg-A positive endocrine cells in the crypts of normal colonic epithelium contain VEGF in their granules and go on to suggest that VEGF may have a role in the maintenance and control of the permeability of the capillary system around the mucosal glands.

Neuropilin-1 is expressed in human colon adenocarcinoma (Parikh *et al.* 2004; Kamiya *et al.* 2006; Ochiumi *et al.* 2006). This was first reported by Parikh *et al.* (2004) who demonstrated with immunohistochemical staining that the NRP1 protein was expressed in all 20 adenocarcinoma specimens studied. Immunofluorescent double staining for NRP1 and CK-22 (an epithelial cell marker) confirmed NRP1 expression was localized to the epithelium. This study also showed that overexpression of NRP1 in human colon adenocarcinoma cells led to a significant increase in tumour growth and tumour vessel count in transfected mice, suggesting that NRP1 is associated with growth and development of colon adenocarcinoma as well as angiogenesis *in vivo*. Hansel *et al.* (2004) demonstrated that the intensity and area of NRP1 expression increase with histological progression from high-grade dysplasia to invasive carcinoma; however, further immunohistochemical analysis of NRP1 in normal and adenomatous tissue has revealed a profound difference in expression pattern. Staining changed from higher intensity in singly dispersed cells in the normal tissue to lower intensity staining in large sections of epithelial cells in adenomas, suggesting NRP1 is dysregulated early in the adenoma-carcinoma sequence (Yu *et al.* 2011). High levels of NRP1 staining in human colorectal carcinoma tissues result in increased proliferation and decreased apoptosis, suggesting that NRP1 may protect cancer cells from apoptosis (Ochiumi *et al.* 2006). Increased NRP1 expression correlates with progression to metastatic disease and prognosis (Ochiumi *et al.* 2006), suggesting that NRP1 expression may aid the identification of patients who would benefit from adjuvant chemotherapy. Although these studies provide strong evidence for NRP1 expression being elevated in colon carcinoma, there is a single contrary report indicating that preserved expression of NRP1 may be associated with a better prognosis (Kamiya *et al.* 2006). This association, however, fails to reach statistical significance and was not independent of disease stage.

Using immunoperoxidase staining, Gray *et al.* demonstrated that NRP2 expression was elevated in most of the human primary and metastatic colon cancer specimens tested compared with normal colonic mucosa. Inhibition of NRP2 with shRNA leads to a decrease in the phosphorylation and activation of VEGFR1 in colorectal cancer cells with a reduction of anchorage independent growth, motility, invasiveness and survival of tumour cells (Gray *et al.* 2008).

As in the upper GI tract, the population of NRP2 expressing cells in the normal colon coincide with a subpopulation of serotonin-producing enteroendocrine cells, with a complete loss of NRP2 expression in enteroendocrine cells derived from carcinoid tumours of the colon, rectum and appendix, despite the tumour cells maintaining their ability to produce and excrete serotonin (Cohen 2001). This had led to speculation that the loss of NRP2 may aid the development of carcinoid tumours, with the NRP2 ligand and tumour suppressor SEMA3F, which has been found to be expressed in the mucosal folds of the developing murine intestine, potentially playing an inhibitory role in tumour development.

Haixia *et al.* (2010) demonstrated mRNA expression of NRP-1, VEGF and SEMA3A in colorectal adenocarcinoma cell lines, with increased ratio of expression of VEGF/SEMA3a ratio when compared to other tumour cell lines. A reduction in SEMA3B mRNA in colorectal carcinomas compared with normal tissue has been reported (Pronina *et al.* 2009). Reports suggest that SEMA3A may contribute to the progression of colon cancer (Nguyen 2006; Muller *et al.* 2007). This may be explained by the interaction of SEMA3A and VEGF, with the dysregulation of SEMA3A expression causing VEGF-driven growth of cancer cells (Catalano *et al.* 2004). Straub *et al.* (2008) identified colonic epithelial cells as the major source of SEMA3C in patients with Crohn's disease. In most of these patients, SEMA3C staining appeared in the basolateral part of the crypt. Patients with

Crohn's disease, irrespective of macroscopic inflammation, had an increased percentage of SEMA3C-positive crypts, whereas control subjects had higher densities of SEMA3C-positive crypts.

The urinary tract

Kidney. Little is known on the role of NRPs in the kidney. Neuropilin-1 and especially NRP2 are significantly more abundant in embryonic rat and mouse kidneys than in newborn or adult kidneys (Villegas & Tufro 2002). Neuropilin-1 is expressed in the developing glomerulus (Robert *et al.* 2000) and in normal human renal glomerular epithelial cells (podocytes) and collecting tubules (Villegas & Tufro 2002). Human podocytes are known to express NRP1 alongside VEGF (predominantly $VEGF_{165}$) (Harper *et al.* 2001). VEGF is crucial for normal glomerular development (Eremina & Quaggin 2004) and is also thought to be protective against nephrotoxic agents, acting as a survival factor, allowing renal tubular cells to survive and proliferate under conditions of extreme stress (Kanellis *et al.* 2000). Semaphorins have also been implicated in nephrogenesis. SEMA3A has been shown to regulate endothelial cell number and podocyte differentiation during glomerulogenesis (Reidy *et al.* 2009) and inhibit ureteric bud branching (Tufro *et al.* 2008). Deletion of plexin-B2, a SEMA receptor that is expressed in pretubular aggregates and the ureteric epithelium in the developing kidney, results in renal hypoplasia and occasional ureteric duplication (Perala *et al.* 2010).

Vascular endothelial growth factor-A has been shown to play a critical role in both the establishment and maintenance of the glomerular filtration barrier (Eremina & Quaggin 2004), although the balance of SEMA3A to VEGF-A may be important in glomerular filtration barrier homoeostasis. In murine studies, exogenous semaphorin3A caused acute nephrotic range proteinuria and decreased VEGF-A receptor expression. However, when $VEGF_{165}$ was administered at the same time as SEMA3A, no proteinuria or renal ultrastructural abnormalities occurred (Tapia *et al.* 2008). Further investigations by Foster *et al.* (2003), examining the physiological role of VEGF at the glomerulus, indicated that VEGF may also act as an autocrine factor on calcium homoeostasis and cell survival; however, the receptor and intracellular regulatory pathways remain to be determined. It has also been suggested that VEGF might induce renal epithelial cell morphogenesis in a NRP1-dependent manner (Karihaloo *et al.* 2005). Moreover, it has also been shown that advanced glycation end-products suppress NRP1 expression in mouse podocytes and that NRP1 expression is decreased in glomeruli of diabetic *db/db* mice when compared with their non-diabetic littermates (Bondeva & Wolf 2009). Both NRP1 and NRP2 were found to be decreased in renal biopsies from patients with diabetic nephropathy when compared with transplant donors (Bondeva *et al.* 2009). Zhou *et al.* (2007) demonstrated a significant decrease in the percentage of NRP1-positive cells among lymphocytes found in rejected kidney graft biopsies, suggesting a potential role of NRP1 as a marker of regulatory T (Treg) cells, enabling prediction of the immune status of kidney grafts.

Schramek *et al.* (2009) suggest a differential role of the two neuropilin isoforms in focal segmental glomerulosclerosis, demonstrating an upregulation of tubular and interstitial NRP2, but not NRP1. In this study, the effect of pro-fibrotic cytokines on NRP expression in human proximal tubular cells was measured. Oncostatin M stimulated the expression of both NRP-1 and NRP2 with transforming growth factor-β1 (TGF-β1), and interleukin-1β (IL-1β) induced upregulation of NRP2 expression but downregulation of NRP-1 expression. They added that a renal biopsy with increased expression of NRP2 mRNA may predict poor renal outcome in nephrotic diseases. Data from Korgaonkar *et al.'s* (2008) study demonstrated that HIV infection stimulates VEGF production in podocytes, with upregulation of NRP1 and VEGFR-2, and downregulation of SEMA3A, contributing to podocyte proliferation.

Cao *et al.* (2008) examined several renal cell carcinoma (RCC) cell lines and found NRP1 expression to be significantly elevated in higher grade compared with lower-grade RCC. A high level of NRP1 expression in RCC was associated with cell migration, invasion and *in vivo* tumour growth. When implanted in mice, RCC cells with a reduced NRP1 level had a significantly smaller tumour forming ability than control cells. This study also showed that NRP1 acts to maintain an undifferentiated phenotype in cancer cells. This was demonstrated with increased expression of epithelial-specific and kidney-specific cadherins in NRP1 knock-down RCC cells, indicating a more differentiated phenotype. Studies have also demonstrated a reduction in SEMA3B gene expression in RCC cell lines (Pronina *et al.* 2009), and that plexin-B1 is downregulated in RCC (Gomez Roman *et al.* 2008).

Bladder. Neuropilin-1 and NRP2 are strongly expressed in human and mouse bladder urothelium, present in the luminal surface and in proximity to the nuclei of the cells (Cheppudira *et al.* 2008; Saban *et al.* 2008a,b, 2010). Neuropilin-2 mRNA was first reported as being strongly expressed in mouse bladder detrusor muscle on embryonic day 15.5 (Chen *et al.* 1997). Saban *et al.* (2008a) determined co-localization of NRP1 with VEGFR-2 and NRP2 with VEGFR-1 in bladder urothelial and ganglia cells. Therefore, it is unsurprising that NRPs are strongly expressed in urothelium cell lines (Saban *et al.* 2008b) and NRP2 expression correlates with advanced tumour stage and grade in bladder cancer (Sanchez-Carbayo *et al.* 2003).

Increasing data from recent studies suggest that the VEGF-NRP pathway may play an important role in the pathogenesis of bladder inflammation, including interstitial cystitis and painful bladder syndrome (Saban *et al.* 2008a). Bladder biopsies from patients with interstitial cystitis, demonstrating glomerulations following hydrodistension, demonstrated increased expression of VEGF protein (Tamaki *et al.* 2004). Upregulation of NRP1, NRP2, VEGFR-2 and

VEGFR-1 was seen in the urothelium of mice with PAR-activated peptide induced bladder inflammation (Saban *et al.* 2008a), and Cheppudira *et al.* (2008) demonstrated that NRP1 and NRP2 expression was significantly increased in chronic, when compared to acute, cyclophosphamide-induced cystitis. Furthermore, there seems to be a more complex role of NRPs in chronic bladder inflammation, with the expression of NRP2 and VEGFR-1 being significantly downregulated in interstitial cystitis compared with control subjects (Saban *et al.* 2008b). In the control bladders, VEGFR-1 and NRP2 were expressed predominantly in the apical cells, whereas in patients with interstitial cystitis, VEGFR-1 and NRP2 were expressed throughout the urothelium. Intra-vesical Bacillus Calmette-Guérin (BCG) instillation in mice increased overall accumulation of VEGF and increased the expression of VEGF and its receptors, VEGFRs and NRPs. This may explain why a subset of patients with interstitial cystitis benefit from BCG therapy.

Neuropilin-2 has also been shown to be strongly expressed in bladder lymphatics. BCG treatment was found to stimulate lymphangiogenesis with increased expression of NRP2. Saban *et al.* (2010) also demonstrated that the systemic administration of NRP1 neutralizing antibodies (anti-$NRP1^A$, which blocks the SEMA domain, and anti-$NRP1^B$, which blocks the VEGF domain) reduced the uptake of VEGF in the bladder of mice receiving intravesical BCG. Both anti-NRP1 antibodies prevented the BCG-induced increase in lymphatic vessel density. Anti-$NRP1^B$ significantly reduced blood vessel density, and anti-$NRP1^A$ was also seen to reduce the accumulation of inflammatory cells. These findings suggest the involvement of semaphorins, as well as VEGF, in the inflammatory response in the bladder. Semaphorin co-receptors, plexin-A2 and A1, have also been demonstrated in bladder mucosa. Microarray gene expression profiles demonstrating the *SEMA3D* gene in bladder epithelium in experimental idiopathic cystitis (Tseng *et al.* 2009) support recent insights that have identified a basic neural pathway that can monitor and adjust the inflammatory response (Tracey 2002), suggesting that SEMA-mediated axonal guidance may have a role in the parasympathetic inflammatory reflex.

The discovery of functionally active VEGF receptors in the urothelium suggests that VEGF-NRP signalling may serve a protective function in inflammatory conditions of the bladder. Chronic bladder inflammation is associated with abnormal capillary growth (Rosamilia & Dwyer 2000) and by uncoupling endothelial cell–cell junctions, VEGF causes vascular permeability (Weis & Cheresh 2005) which may correspond to the 'leaky' urothelium seen in interstitial cystitis. Interestingly, it has also been suggested that the hypothesis for a connection between neural and epithelial function (Apodaca *et al.* 2003) could potentially be modulated by neuropilins.

Prostate. Neuropilins, VEGF and semaphorins are expressed in normal prostate epithelium (Jackson *et al.* 2002; Yacoub *et al.* 2009). Immunohistochemical examination of normal prostate tissue reveals a relatively low focal expression of NRP1 on the membrane of luminal epithelial cells, when compared to diffuse expression of SEMA3A, with cytoplasmic and membranous immunoreactivity (Yacoub *et al.* 2009). VEGF expression is sparse and present in under one-third of cases and located in the cytoplasm of basal cells (Jackson *et al.* 2002). VEGFR-1 and R-2 immunoreactivity were found to be either weak or not detected in normal prostatic epithelium.

Prostate cancers express high levels of NRP1, at both mRNA and protein level (Latil *et al.* 2000; Vanveldhuizen *et al.* 2003; Yacoub *et al.* 2009), with overexpression shown to be associated with higher Gleason grade, more advanced stage, increased metastatic potential in prostate carcinoma and overexpression of VEGF (Latil *et al.* 2000). Increased expression of NRP2 in human prostate cancer cell lines has also been observed (Muders *et al.* 2009). Yacoub *et al.* (2009) concluded that opposite autocrine loops involving NRP1 and both the 'anti-tumoural' SEAM3A and the 'pro-tumoural' VEGF may well play a key role in disease progression in prostate cancer. In this study, co-expression of NRP1 and SEMA3A in prostate cancer cells was associated with good prognosis, including lower prostate-specific antigen (PSA), grade and stage, and VEGF expression was mainly found in poor prognosis disease. In clinically localized and hormone-naïve prostate cancer, NRP1 expression was significantly associated with SEMA3A expression and not VEGF expression. In hormone-refractory prostate cancer, no relationship was seen between NRP1 and these two ligands.

Respiratory tract

Neuropilin-1 is expressed in normal alveolar epithelium (Ito *et al.* 2000; Roche *et al.* 2002). Neuropilin-1 levels increase during lung organogenesis (Roche *et al.* 2002), and ligands SEMA3A and VEGF contribute to alveolar septation (Gerber *et al.* 1999; Ito *et al.* 2000; McGrath-Morrow *et al.* 2005). SEMA3A inhibits branching morphogenesis in lung bud organ cultures, acting via NRP1 (Ito *et al.* 2000), whilst SEMA3C and SEMA3F have been found to promote lung branching morphogenesis via both NRP1 and NRP2 (Kagoshima & Ito 2001). Expression of SEMA3F has been observed in the membrane of type I and II epithelial cells in normal human lungs (Favre *et al.* 2003).

The expression of NRP and its ligands in lung cancer is widely reported. Neuropilin-1 and NRP2 are overexpressed in lung cancer (Kawakami *et al.* 2002). A progressive upregulation of NRP levels are observed from benign bronchial hyperplasia to dysplasia and then invasive carcinoma (Lantuejoul *et al.* 2003). High levels of NRP1 expression correlate with shorter disease-free and overall survival, and combined overexpression of NRP1 and NRP2 is associated with a worse prognosis than when either NRP is singly overexpressed (Kawakami *et al.* 2002). A single report has also observed overexpression of NRP1 in laryngeal carcinoma (Zhang & Kong 2006).

The majority of studies on semaphorins in lung cancer focus on the tumour suppressors SEMA3B and SEMAF. SEMA3B transfection stimulates apoptosis and inhibits lung cancer cell growth. SEMA3F has been shown to inhibit lung cancer cell growth with lower integrin activation, reduced MAPK signalling and loss of HIF-1α expression and VEGF secretion. Favre *et al.* (2003) demonstrated in human lung cancer cells that SEMA3F, which is normally located in the membrane of epithelial cells, is lost or delocalized into the cytoplasm. Loss of SEMA3F correlates with increased VEGF staining in the cell membrane, suggesting competition for the NRP receptors. In lung cancer, SEMA3F staining correlates inversely with tumour stage. In contrast, SEMA3C has been found to be upregulated in lung cancer cells with higher metastatic potential, suggesting that SEMA3C may be an inducer of tumour progression (Nasarre *et al.* 2010).

Vascular endothelial growth factor-deficient mice were found to have spontaneous airspace enlargement (Serpa *et al.* 2010), and *in vitro* studies suggest VEGF has a role in preservation of alveolar cell survival (Kasahara *et al.* 2000). VEGF also reduces lung epithelial cell apoptosis *in vitro* following induced hydrogen peroxide injury (Roberts *et al.* 2007), proposing VEGF as a potential therapy in acute respiratory distress syndrome. Pulmonary epithelial NRP1 deletion also results in increase in airspace size and enhances the susceptibility of type I and type II alveolar epithelial cells to cigarette smoke-induced apoptosis (Le *et al.* 2009). Human data demonstrate reduced expression of NRP1 protein in the lungs of smokers with chronic obstructive pulmonary disease (Marwick *et al.* 2006). These studies support a role for NRP1 in development and homoeostasis of normal alveolar epithelium.

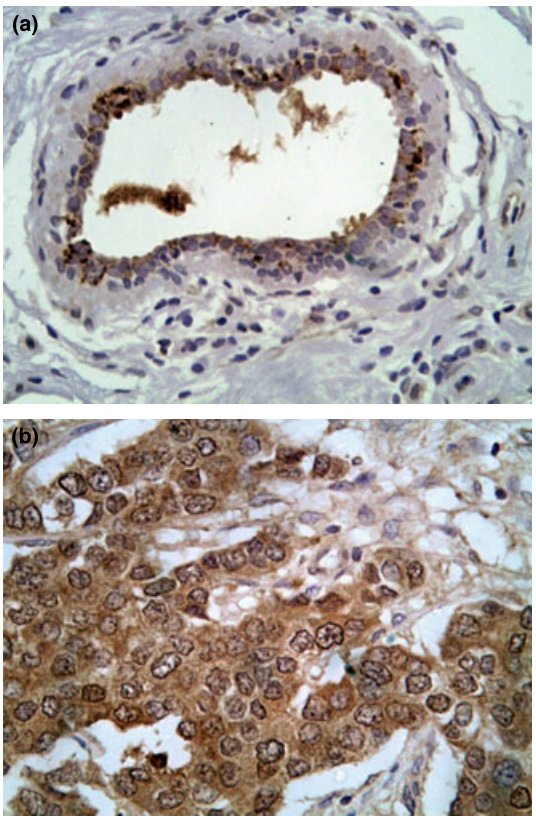

Figure 7 Immunohistochemical staining demonstrating (a) NRP1 expression in normal breast ductal epithelial cells and (b) NRP2 expression in invasive breast carcinoma. (×40 objective lens, Author's own photograph [CS]).

Breast

Expression of both NRP1 and NRP2 has been demonstrated in normal and neoplastic breast epithelium (Figure 7) with NRP2/VEGF ligation also found to contribute towards branching morphogenesis of mammary epithelial cells in a murine model (Goel *et al.* 2011). Stephenson *et al.* (2002) demonstrated the expression and distribution of NRP1 in normal and preneoplastic breast tissue using RT-PCR and immunohistochemical analysis revealing membranous and cytoplasmic NRP1 expression in normal ductal epithelium, with expression increasing from normal to premalignant (atypical ductal hyperplasia) and preinvasive (ductal carcinoma *in situ*) lesions (Staton *et al.* 2011).

Both NRPs are expressed in breast cancer cells (Stephenson *et al.* 2002; Bachelder *et al.* 2003), with expression correlating with poor prognosis (Ghosh *et al.* 2008; Yasuoka *et al.* 2009) and NRP2 expression associated with lymph node status (Yasuoka *et al.* 2009). In invasive cancer, when compared with normal ductal epithelium, the expression of NRP1 in tumour cells has been shown to decrease with differential expression patterns, with expression of NRP2 found not to change with lesion severity (Staton *et al.* 2011). In this same study, expression of SEMA3A, SEMA3B, SEMA3F, plexin-A1 and plexin-A3 was demonstrated in normal epithelium; however, their expression decreased with increasing severity lesion, indicating potential tumour suppressor activity. SEMA3A expression was found to be restricted to the normal myoepithelium. With invasive cancer known to lack myoepithelial cells, it is unsurprising that SEMA3A expression is completely absent in such lesions. This contrasts with SEMA3A expression in lung and ovarian cancer, where although expression decreases, it remains present in cancerous lesions. Likewise, SEMA3F expression, which is restricted to the luminal epithelium of a few ducts in normal breast tissue, becomes absent in invasive breast cancer. Data have revealed that SEMA3A inhibits breast cancer cell migration and spreading *in vitro* (Bachelder *et al.* 2003; Herman & Meadows 2007). SEMA3F expression in breast carcinoma cells inhibits their adhesion and spreading, which is potentially mediated by loss of E-cadherin (Nasarre *et al.* 2005). Plexin-A1 (Bachelder *et al.* 2003; Castro-Rivera *et al.* 2004) and plexin-B1 (Rody *et al.* 2007) expressions have also been demonstrated in breast cancer cells with loss of plexin-B1 expression associated

with poor outcome in oestrogen receptor-positive breast cancer (Rody *et al.* 2007).

Again reflecting NRP expression, VEGF is also expressed in the cytoplasm of normal epithelial ductal cells (Viacava *et al.* 2004; Bluff *et al.* 2009) with an upregulation in hyperplastic epithelium when compared with normal cells (Pavlakis *et al.* 2008; Bluff *et al.* 2009). Increased expression of VEGF, VEGFR-1 and VEGFR-1 in invasive breast carcinoma is well documented (Ghosh *et al.* 2008) and immunohistochemical expression correlates with prognosis (Toi *et al.* 1995). The SEMA3A/VEGF ratio also correlates with the chemotactic rate of breast cancer cells (Bachelder *et al.* 2003).

Ovary

Although an early study was unable to identify NRP1 expression (Baba *et al.* 2007), more recently, NRP1 and NRP2 have been found to be weakly expressed in normal ovarian epithelium (Drenberg *et al.* 2009). Both NRPs are also expressed in the stroma of normal ovaries, with NRP2 demonstrating stronger staining. In Drenberg *et al.'s* (2009) study the majority of normal ovarian surface epithelium expressed NRP2, with both cytoplasmic and membranous staining. In contrast, NRP1 is overexpressed in ovarian epithelial cancer cells, with predominantly cytoplasmic staining (Baba *et al.* 2007). The percentage of epithelial cells expressing NRP1 increases with disease progression, whereas expression of NRP2 was found to decrease with progression of epithelial ovarian cancer (Drenberg *et al.* 2009). SEMA3F is expressed in normal ovarian epithelium, also with staining in normal and neoplastic epithelium being predominantly cytoplasmic with a small proportion demonstrating basal membranous staining (Drenberg *et al.* 2009). SEMA3A, SEMA3B and SEMA3F expressions decrease with disease progression in ovarian carcinoma (Osada *et al.* 2006). Hall *et al.* (2005) demonstrated increased expression of VEGF in the epithelium of malignant ovarian lesions, which co-localized with somatostatin expression in the epithelium. As with breast carcinoma, patients with ovarian carcinoma with a high VEGF/SEMA ratio have a worse prognosis, compared with those with lower VEGF/SEMA ratio.

Epidermis

Man *et al.* (2006) have demonstrated that keratinocytes in the normal epidermis express both NRP1 and NRP2 at both mRNA and protein levels. Neuropilin-1 and NRP2 are expressed in the membrane and cytoplasm of keratinocytes in all but the stratum corneum layer in the normal epidermis. Immunostaining has also identified VEGFR-1, VEGFR-2 and VEGFR-3 in normal keratinocytes (Wilgus *et al.* 2005; Man *et al.* 2006) and exogenous VEGF treatment has been observed to increase the proliferation and migration of normal keratinocytes (Man *et al.* 2006), suggesting that NRPs and VEGFRs possibly have an autocrine signalling role in the epidermis. Neuropilin-1, NRP2, VEGFR2 and VEGF are overexpressed in psoriasis (Detmar *et al.* 1994; Henno *et al.* 2010), and NRP1 and NRP2 are also expressed in malignant melanoma (Lacal *et al.* 2000; Straume & Akslen 2003; Bielenberg *et al.* 2006; Rushing *et al.* 2011). Neuropilins have a role in wound repair; however, expression is confined to endothelial cells, fibroblasts and a few macrophages (Kumar *et al.* 2009).

SEMA3A is also expressed in keratinocytes in normal epidermis. Expression of SEMA3A is reduced in patients with atopic dermatitis (AD) when compared to healthy controls (Tominaga *et al.* 2008) with intracutaneous injection of recombinant SEMA3A being shown to improve the skin lesions of mice in an animal model of AD with a decrease in epidermal thickness and density of invasive nerve fibres in the epidermis (Yamaguchi *et al.* 2008).

Other epithelial cell types

Neuropilin-1, NRP2 and SEMA3F are expressed in the developing parathyroid and thymus, and there is emerging evidence demonstrating a role for NRP1 in T-cell function with NRP1 expressed on thymic epithelial and dendritic cells implicated in T-cell activation and regulation (Sarris *et al.* 2008; Takamatsu *et al.* 2010). Neuropilin-1 also regulates SEMA3A-mediated thymocyte migration (Lepelletier *et al.* 2007) and, acting as a receptor for TGFβ1, activates latent TGFβ1in T-cells. Neuropilin-2 is also overexpressed in papillary thyroid cancer (Finley *et al.* 2004). There is a single report of NRP2 expression in salivary adenoid cystic carcinoma (Cai *et al.* 2010), with expression again correlating with advanced clinical stage and poor prognosis. Pro-angiogenic factors are known to play an important role in the neovascularization associated with age-related macular regeneration (AMD) with recent advances in anti-VEGF therapies shown to preserve and improve visual acuity (Ciulla & Rosenfeld 2009), NRP1 has also been found to be expressed in the retinal pigment epithelial cells of surgically excised choroidal neovascular membranes and is also thought to play a role in choroidal neovascularization in AMD (Cui *et al.* 2003; Lim *et al.* 2005).

Neuropilins as therapeutic targets and translational advances

As a result of overexpression of NRP in the majority of carcinomas, there is increasing interest in NRP as therapeutic target. Neuropilin antagonists include anti-NRP1 antibodies, semaphorins, sNRP1 and $VEGF_{165}$- and NRP-derived peptides that block the $VEGF_{165}$-NRP interaction (Geretti & Klagsbrun 2007). Strategies that target VEGF/NRP and VEGF/VEGFR2 interactions are summarized in Figure 8. To date, the majority of anti-angiogenic therapies have been developed to target the VEGF/VEGFR pathway (Eichholz *et al.* 2010) with the anti-VEGF monoclonal antibody bevacizumab, used in combination with standard chemotherapy, improving survival in metastatic colorectal cancer (Hurwitz *et al.* 2004) and progression free survival time in metastatic lung cancer (Sandler *et al.* 2006) and metastatic renal cell

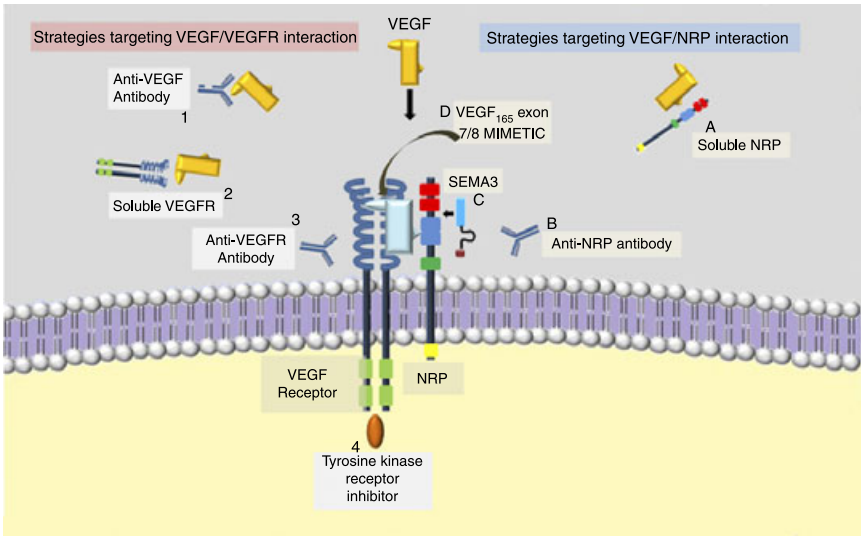

Figure 8 Strategies targeting the vascular endothelial growth factor (VEGF)/VEGFR and VEGF/NRP interactions. Antagonists of the VEGF/VEGF receptor interaction include: 1) anti-VEGF antibodies, such as bevacuzimab and ranimizumab (Genentech/Roche), 2) soluble VEGFRs, such as Aflibercept (Sanofi-Aventis), 3) Anti-VEGFR antibodies, such as ramucirumab (IMC-1121B – Imclone Systems), a recombinant humanized IgG1 monoclonal antibody specifically against VEGFR-2 and 4) tyrosine kinase inhibitors, such as sorafenib (Bayer) and sunitinib (Pfizer), that compete with ATP for binding to the catalytic site of receptor tyrosine kinases. Strategies targeting the VEGF/NRP interaction include: (A) soluble Neuropilin-1 (NRP1) (sNRP1), (B) anti-NRP antibodies, such as anti-NRP1^{B}, which specifically blocks the –b domain, enhancing the anti-tumour effects of bevacizumab in an animal model, (C) Class 3 semaphorins are angiogenesis and tumour growth inhibitors, in particular SEMA3A, which inhibits VEGF164 induced EC motility, (D) Peptides that correspond to VEGF exon 7 and 8 also interfere with NRP ligation withVEGF.

carcinoma (Escudier *et al.* 2007b). The benefit in metastatic breast cancer remains controversial with the initial FDA approval having now been removed following further trials (Petrelli & Barni 2010). VEGFR tyrosine kinase inhibitors, sorafenib and sunitinib, have been approved for the treatment of advanced renal cell carcinoma (Escudier *et al.* 2007a), unresectable HCC (Llovet *et al.* 2008) and gastrointestinal stromal tumours (Demetri *et al.* 2006). Aflibercept, a soluble receptor that binds directly to VEGF, is currently being tested in phase III trials for use in combination for the treatment of metastatic colorectal, non-small cell lung and androgen-independent prostate cancer (Eichholz *et al.* 2010).

Limited efficacy and resistance associated with current anti-angiogenic therapies do, however, remain problematic. Inhibition of VEGF binding by an anti-NRP1^{B} antibody, which specifically blocks the –b domain, enhances the anti-tumour effects of the anti-VEGF antibody bevacizumab in a mouse model (Pan *et al.* 2007). Anti-NRP^{B} antibody also enhances chemosensitivity by interfering with integrin-dependent survival pathways (Jia *et al.* 2010). These findings have led to attractive speculation that the combination of anti-NRP1 with anti-VEGF agents could improve patient survival in advanced malignancy. The safety profile of the human monoclonal anti-NRP1 antibody MNRP1685A is now currently being assessed in phase 1b trial in combination with bevacizumab with or without paclitaxel in patients with locally advance or metastatic solid tumours (Genentech 2009). Anti-NRP1^{A} antibodies, which specifically block semaphorin binding to the –a domain, have been shown to reduce the accumulation of inflammatory cells in a model of chronic bladder inflammation (Saban *et al.* 2010). Overexpression of sNRP1 reduced apoptosis in prostate cancer cells (Gagnon *et al.* 2000) and also inhibited breast cancer cell migration (Cackowski *et al.* 2004). sNRP1 is thought to act by blocking $VEGF_{165}$ binding leading to sequestration of VEGF and thereby reducing its angiogenic and tumorigenic effects (Geretti & Klagsbrun 2007). $VEGF_{165}$ binds exclusively to the NRP –b domain (Mamluk 2002). This therefore allows selective binding of a NRP –b domain peptide to target $VEGF_{165}$ and not SEMA3A.

Other strategies developed to inhibit $VEGF_{165}$-NRP1 interaction include peptides and analogues corresponding to VEGF exons 7 and 8 (Soker *et al.* 1997; von Wronski *et al.* 2006) and the NRP1 binding site (Jia *et al.* 2006). The dietary fibre fermentation product butyrate has also been found to downregulate NRP1 and VEGF in colorectal cancer cell lines (Yu *et al.* 2010), and faecal butyrate levels are inversely proportional to NRP1 *in vivo*, suggesting a novel contributory mechanism to the chemopreventive effect of dietary fibre (Yu *et al.* 2011). Anti-NRP2^{B} antibodies also inhibit the formation of tumour lymphatics in a mouse model (Caunt *et al.* 2008). Although such anti-lymphangiogenic treatment strategy is yet to be clinically assessed, there is increasing evidence that blocking NRP2 leads to a reduction in functional tumour lymphatics, providing an attractive prospect for modulating metastasis. Recent studies indicating NRP as a promoter of EMT, a critical step in tumour invasion and disease progression, adding further evidence that

NRP is involved in multiple oncogenic functions and therefore an attractive target for anti-tumour therapy. Another potential research avenue to pursue is that of dual inhibition of tumours with both NRP1 and NRP2 which may provide additional benefit as a combination therapy. In addition to NRP's potential as an anti-cancer agent, recent findings indicate that NRP may also influence fibrosis via PDGF and TGF-β1 signalling and therefore, with angiogenesis and VEGF also shown to have important roles in fibrosis (Yoshiji *et al.* 2003), the potential of targeting NRP1 to 'hit three birds with one stone' as an antifibrotic agent has also been suggested (Troeger & Schwabe 2011).

Conclusion and future direction

The importance of NRPs in the development of the nervous and cardiovascular systems and in angiogenesis is well established. There is mounting evidence implicating NRPs in alternative roles in tumour biology, such as modulating the balance between cell proliferation and survival. There is therefore increasing evidence supporting targeting the NRP pathway in neoadjuvant and adjuvant cancer therapy, although the mechanisms surrounding NRPs remain incompletely understood.

The focus of studies on expression of NRPs and their ligands in the epithelium has been carcinoma, and there are comparatively few studies describing such expression in normal epithelium. Where these studies have been carried out, consistent patterns have emerged of NRP expression in normal epithelium, in singly distributed subpopulations often associated with endocrine activity. With the discovery of novel ligands and signalling mechanisms, it is anticipated that NRPs may have a far wider spectrum of activity than is currently appreciated. Specific cellular subtypes that express NRP have, however, yet to be established, and hence, the precise role of NRP in epithelium remains undetermined.

There is increasing interest in the biological roles of VEGF in non-angiogenesis-related cellular function, and likewise, future NRP research must be directed towards their role in normal physiological tissues and to establish the extent or otherwise to which endothelial signalling mechanisms are replicated in the epithelium. Dysregulation of NRP expression in epithelial cells is a common feature of cancer and appears to be a very early event. With roles in angiogenesis, apoptosis and EMT, NRP may prove an attractive target in specific and multiple cancer processes.

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